Some Aspects of the Behavior of Mosquito Larvae (Diptera: Culicidae)[1]

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ABSTRACT

This review of the recent literature dealing with behavior of mosquito larvae emphasizes newer knowledge of feeding habits, orientation to the water surface, reactions to physical stimuli, formation of aggregations or clusters, and effects of overcrowding. Of particular interest are growth retardant factors produced by larvae of a given species which play a part in competitive displacement.

In recent years considerable attention has been given to the eggs of mosquitoes with particular reference to quiescence, diapause, and hatching stimuli. Much research has been conducted on adult behavior, with special emphasis on feeding, mating, oviposition, and flight. Not so much consideration has been given to the behavior of larvae. Clements (1963) reviewed the subject very briefly.

The larva, for all practical purposes, is unable to choose its environment. Its mother has that responsibility. It can move only short distances to select slightly different conditions of temperature and light, to reach food sources, or to evade enemies. Because predaceous larvae are relatively rare they will not be considered here. Larvae of various species have evolved adaptations to many different kinds of water. Variations in the nature of the aquatic environment, for the most part, involve the following factors: temperature, light, movement of water, dissolved gases, hydrogen-ion concentration, organic matter, and inorganic salts. Each of these may have an effect indirectly as well as directly. For example, shade may influence the growth of micro-organisms which constitute the larval diet. Quantities of salts which larvae will tolerate are known for several species.

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According to Bates (1949), food is rarely a limiting factor. That is, competition for food is hardly ever intense, although larvae in small containers at times are affected by food shortages. Young larvae are said to feed primarily on bacteria. Older larvae take in larger micro-organisms such as algae, yeasts, fungi, and protozoa. They also swallow small particles of organic matter. Laboratory investigations by several workers show that suboptimum amounts of food cause an increase in the duration of larval and pupal stages and a decrease in size and weight of adults. Larvae of *Aedes aegypti* were reared under sterile conditions by Trager (1935). He was able to dissolve the right combination of nutrients and vitamins in water so that the larvae in his experiments swallowed sufficient quantities of the medium to develop to maturity. The surfaces of eggs were sterilized, and the medium was kept entirely free of micro-organisms. Similar procedures have been carried out by several other workers using a few different species. Recently Wallis and Lite (1970) reported on the axenic rearing of *Culex salinarius*. Vitamins are an essential part of an artificial diet. In nature bacteria and other micro-organisms appear to produce growth-stimulating substances.

Normally larvae use their mouth brushes to filter out particulate matter. *Anopheles* larvae which remain at the surface create eddies and tend to suck so that currents at the surface film move toward them (Renn, 1941). This facilitates the filtering out of particles resting on the surface. Surtees (1959) classified non-predaceous larvae as either filter feeders or browsers. Many culicines are filter feeders and produce currents below the water surface. Browsers are usually bottom feeders. The brushes of their mouth parts are shorter and stiffer than those of filter feeders. Browsers abrade solid material and manipulate fairly large particles, breaking them down to smaller sizes so that they can be swallowed. For example, they break loose clusters of micro-organisms clinging to large pieces of debris. They frequently are seen browsing on their own bodies. According to Pucat (1965), filter feeders may feed on particles stirred up by browsers.

Locomotion of larvae depends largely on body jerks, but the mouth brushes are used for pulling the body along. The ventral brush is used as a sculling organ (Ross, 1951), and it probably serves at times as a rudder.

Larvae are sensitive to changes in light, to vibrations, and to differences in temperature. Nearly all of them are heavier than water. When they are at the surface they respond to shadows moving across the water or to vibrations by sinking to the bottom of the medium. These alarm reactions were studied in some detail by Folger (1946), Thomas (1950), and Mellanby (1958). "Crash diving" is not demonstrable in some species but is well developed in many *Anopheles* and other species which spend most of the time at the water surface. Some species are more responsive to vibrations than to changes in light intensity. Mellanby (1958) showed that *Aedes aegypti* larvae can be conditioned or habituated to the rapid repetition of a stimulus that initially causes an alarm response. Crash-diving results when the side of a dish containing *A. aegypti* larvae is tapped, and the larvae stay at the bottom for 4 minutes. But if the container is tapped once every second the larvae, in effect, ignore the tapping. One may reasonably ask if these larvae go through a learning process! Contitioning to light changes has also been observed.

One of my students, Shahin Navai, is currently studying responses of *Aedes atropalpus* larvae to vibrations and has found that they behave somewhat like *A. aegypti* larvae. However, tapping the container produces different reactions among larvae which are part of a group of 10 compared with reactions of single larva in a dish. We are now studying the effects of vibrations from a tuning fork, and perhaps we can ascertain whether or not these larvae are capable of "hearing".

Nearly all mosquito workers have observed the "balling" or clustering of large numbers of larvae in a relatively small pool or in a container. Hocking (1953) attributed such aggregations to mutual orientation of larvae toward their shadows. Detailed studies of *Aedes taeniorhynchus* larvae by Nayar and Sauerman (1968) have shown that aggregations probably occur in response to visual stimuli and bodily contacts. Aggregations do not occur at night. They are related to temperature and the nutritional state of the larvae, and it is suggested that they aid in the synchronization of pupal ecdysis. A relatively few controlled experiments prove that photo-period, apart from temperature, affects growth rates. Chiba (1968) found that in the case of *Armigeres subalbatus*, which overwinters in the larval stage, long days activate larvae to pupate and short days cause larvae to remain in the 4th instar. Some larvae, of course, complete their development in complete darkness (Bickley, 1954).

There are numerous studies on temperature effects. One of the most interesting concerns the aggregation of larvae in Arctic pools. Clusters of larvae move around small pools in a clockwise direction just as the sunlight strikes the pools. Haufe (1957) measured horizontal and vertical temperature gradients which cause a 3-dimensional displacement of larvae. The balling of larvae was also considered in relation to light and gravity.

Optimum, minimum, and maximum temperatures for a number of species have been established. Larvae of nearly all species are killed when actually frozen. The effects of low temperatures may be ameliorated by a process of acclimatization. In other words, a gradual decline in temperature to a low point is not as detrimental as a sudden change (Mellanby, 1960).

There have been few experiments using water with temperature gradients. *Aedes aegypti* larvae respond to horizontal gradients and select a favorable zone. According to Omardeen (1957), they move to the spot which is less irritating to them.

It is generally recognized that the orientation of culicine larvae to the surface essentially is a result of the need for obtaining air. Temperature, light, and gravity are entirely secondary. Meola (1961) reared larvae of *Aedes aegypti* in culture tubes where the only source of air was from below. Keeping the tail-end down caused no serious problems. One of my students, John B. Duvall, has reared larvae of *Aedes atropalpus* "upside down". We can get the larvae to pupate but have been unable to obtain adults.

Mosquito larvae with few exceptions avoid currents. This, in part, accounts for the fact that larvae are generally absent from open water. The other reason why they shun open water may be attributed to a natural thigmotropism or thigmotaxis. It may be assumed that, as species evolved, the instinct to touch or at least stay close to vegetation or other objects in the water had tremendous adaptive value in protecting larvae from fish and other predators. Even so, it is rather surprising to find that in small ponds elimination of vegetation around the edges generally prevents development of mosquito larvae.

Schober (1966) reported that agitation of the water surface by continuous sprinkling actually killed larvae and pupae of *Culex pipiens* and prevented oviposition. This procedure has practical value in controlling mosquito breeding in lagoons designed for holding organic wastes.

In most aquatic communities mosquito larvae are not dominant members (Bates, 1949). There are certain notable exceptions such as bromeliads, small containers, temporary rain-pools, and certain types of heavily polluted water.

Shannon and Putnam (1934) were among the first to report on the effects of overcrowding of *Aedes aegypti* and on the effect of water "previously fouled" by larvae. In recent years there has been increasing interest in competition among larvae of the same species and between different species. If a given species competes favorably against another species and is dominant, then it is said that that species occupies a particular ecological niche. The less successful species is said to occupy a different ecological niche. It is difficult to explain just how these specific adaptations evolved and why they persist. It now appears that in several cases the most important single factor involved in population regulation or competitive displacement is a substance produced by larvae. Moore and Fisher (1969) have suggested GRF, or growth retardant factor, as the name for this substance. In their studies, *A. aegypti* larvae produced a substance which slowed down the growth of *A. albopictus*. This was proved by placing larvae of *A. albopictus* in water "fouled" by *A. aegypti*. Peters et al. (1969) reported that the presence of *A. aegypti* larvae in a culture of *Culex pipiens* caused significant mortality of the latter species. Barbosa et al. (1972) reared *A. aegypti* larvae at various densities. Overcrowding resulted in decreased survival and pupal weights. Mechanical agitation probably caused a decrease in feeding although there appeared to be evidence that metabolites had an effect on growth. Wada (1965) stated that the detrimental effects of high densities on *A. aegypti* larvae could not be attributed to metabolic wastes. Wilton (1968) found that *A. aegypti* larvae were more efficient in utilizing food than *A. triseriatus* larvae. Consequently *A. aegypti* has a competitive advantage. Ikeshoji and Mulla (1970) reared *Culex pipiens quinquefasciatus* larvae under crowded conditions and found that toxic chemical factors produced by the larvae affected larvae of the same species and larvae of *C. tarsalis*, *A. aegypti*, and *Anopheles albimanus*. Toxic factors were ether extractable, and progress was made in identifying biologically active materials by thin layer chromatography. There is good reason to believe that at some time toxic factors produced by larvae can be further identified and synthesized. These substances may be the larvicides of the future.

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